16:44:37 OCA PAD AMENDMENT - PROJECT HEADER INFORMATION 04/29/93

Active

Project #: E-19-X04 Cost share #: Rev #: 3
Center # : 10/24-6-R7254-2A0 Center shr #: OCA file #:
Work type : RES
Contract#: 5 R29 HL44960-02 Mod #: MEMO OF 4/20/93 Document : GRANT
Prime #: Contract entity: GTRC

Subprojects ? : N CFDA:
Main project #: PE #: N/A

Project unit: CHEM ENGR Unit code: 02.010.114
Project director(s):
WICK T M CHEM ENGR (404)894-8795

Sponsor/division names: DHHS/PHS/NIH / NATL INSTITUTES OF HEALTH
Sponsor/division codes: 108 / 001

Award period: 920701 to 930630 (performance) 930930 (reports)

Sponsor amount	New this change	Total to date
Contract value	0.00	103,324.00
Funded	0.00	103,324.00
Cost sharing amount		0.00

Does subcontracting plan apply ?: N

Title: MECHANISM OF SICKLE ERYTHROCYTE/ENDOTHELIAL ADHESION

PROJECT ADMINISTRATION DATA

OCA contact: Kathleen R. Ehlinger 894-4820

Sponsor technical contact

DR. CLARICE REID
(301)496-6931

NATIONAL HEART, LUNG, AND BLOOD INST
NATIONAL INSTITUTES OF HEALTH
9000 ROCKVILLE PIKE
BETHESDA, MD 20892

Sponsor issuing office

TIJUANNA DECOSTER
(301)496-7257

NATIONAL HEART, LUNG, & BLOOD INST
NATIONAL INSTITUTES OF HEALTH
9000 ROCKVILLE PIKE
BETHESDA, MD 20892

Security class (U,C,S,TS) : U ONR resident rep. is ACO (Y/N): N
Defense priority rating : N/A NIH supplemental sheet
Equipment title vests with: Sponsor GIT X

Administrative comments -
ISSUED TO REVISE DELIVERABLE SCHEDULE.

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 09/24/93

Project No. E-19-X04 Center No. 10/24-6-R7254-2A0

Project Director WICK T M School/Lab CHEM ENGR

Sponsor DHHS/PHS/NIH/NATL INSTITUTES OF HEALTH

Contract/Grant No. 5 R29 HL44960-02 Contract Entity GTRC

Prime Contract No.

Title MECHANISM OF SICKLE ERYTHROCYTE/ENDOTHELIAL ADHESION

Effective Completion Date 930630 (Performance) 930930 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	Y	
Final Report of Inventions and/or Subcontracts	Y	
Government Property Inventory & Related Certificate	N	
Classified Material Certificate	N	
Release and Assignment	N	
Other	N	

Comments EFFECTIVE DATE 7-1-92. CONTRACT VALUE $103,324.

Subproject Under Main Project No.

Continues Project No. E-19-660

Distribution Required:

Project Director	Y
Administrative Network Representative	Y
GTRI Accounting/Grants and Contracts	Y
Procurement/Supply Services	Y
Research Property Managment	Y
Research Security Services	N
Reports Coordinator (OCA)	Y
GTRC	Y
Project File	Y
Other CARL BAXTER-FMD	Y
FRED CAIN-OOD	Y

NOTE: Final Patent Questionnaire sent to PDPI.

Form approved through 6/30/94
OMB No. 0925-0001

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE

APPLICATION FOR CONTINUATION GRANT

REVIEW GROUP	TYPE	ACTIVITY	GRANT NUMBER
	5	R29	HL44960-03

TOTAL PROJECT PERIOD From: 07/25/91 Through: 06/30/96

REQUESTED BUDGET PERIOD From: 07/01/93 Through: 06/30/94

To be verified by applicant. Check information in Items 1 through 6. If incorrect, furnish correct information in Item 13.

1. TITLE OF PROJECT
MECHANISM OF SICKLE ERYTHROCYTE/ENDOTHELIAL ADHESION

2a. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR *(Name and address, street, city, state, zip code)*
WICK, TIMOTHY M
GEORGIA INST. OF TECHNOLOGY
778 ATLANTIC DR

4. APPLICANT ORGANIZATION *(Name and address, street, city, state, zip code)*
GEORGIA TECH RES CORP
OCA/PID RM 246 CRB
GEORGIA INST OF TECHNOLOGY
ATLANTA, GA 30332-0420

BITNET/INTERNET ADDRESS

5. ENTITY IDENTIFICATION NUMBER 158060314 6A1

2b. DEPARTMENT, SERVICE, LABORATORY OR EQUIVALENT
SCHOOL OF CHEMICAL ENGINEERING

2c. MAJOR SUBDIVISION
COLLEGE OF ENGINEERING

3. ORGANIZATIONAL COMPONENT TO RECEIVE CREDIT FOR BIOMEDICAL RESEARCH SUPPORT GRANT *(See instructions)*
20 OTHER

6. TITLE AND ADDRESS OF ADMINISTRATIVE OFFICIAL
CONTRACTING OFFICER
OCA/PID, RM 246 CRB
GEORGIA INST. OF TECHNOLOGY
ATLANTA, GA 30332-0420
3

BITNET/INTERNET ADDRESS timothy.wick@che.gatech.edu

Complete the following *(see instructions)*

7. HUMAN SUBJECTS — If "YES" exemption no. or IRB approval date — 4b. Assurance of compliance no.
[ ] 7a. NO [xx] YES — 3/10/93 — M1395

8. VERTEBRATE ANIMALS — If "YES," IACUC approval date — 8b. Animal welfare assurance no.
[xx] 8a. NO [ ] YES

9. PERFORMANCE SITE(S) *(Organizations and addresses)*
Georgia Institute of Technology
Cellular Biomechanics Laboratory
Space Science and Technology Building A
Room 217
Atlanta, GA 30332-0405

10. COSTS REQUESTED FOR NEXT BUDGET PERIOD
10a. DIRECT $ 10b. TOTAL $

11. INVENTIONS AND PATENTS *(See instructions)*
[xx] NO [ ] YES If "YES," [ ] Previously reported [ ] Not previously reported

TELEPHONE AND FAX INFORMATION

	AREA CODE	TELEPHONE NO. AND FAX NO.
12a. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR *(Item 2a)*	404	894-8795 (phone)
	404	894-2866 (fax)
12b. NAME OF ADMINISTRATIVE OFFICIAL *(Item 6)* Janis L. Goddard	404	894-4817 (Phone)
12c. NAME AND TITLE OF OFFICIAL SIGNING FOR APPLICANT ORGANIZATION *(Item 15)* Janis L. Goddard Contracting Officer	404	894-6956 (Fax)
		894

BITNET/INTERNET ADDRESS

13. USE THIS SPACE FOR CORRECTIONS TO ITEMS 1 THROUGH 6. INDICATE THE NUMBER(S) WHERE ANSWERS APPLY.

None

14. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application. Willful provision of false information is a criminal offense (U.S. Code, Title 18, Section 1001). I am aware that any false, fictitious, or fraudulent statement may, in addition to other remedies available to the Government, subject me to civil penalties under the Program Fraud Civil Remedies Act of 1986 (45 CFR 79).	SIGNATURE OF PERSON NAMED IN 2a *(In ink. "Per" signature not acceptable.)*	DATE
15. CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true and complete to the best of my knowledge, and accept the obligation to comply with the Public Health Service terms and conditions if a grant is awarded as the result of this application. A willfully false certification is a criminal offense (U.S. Code, Title 18, Section 1001). I am aware that any false, fictitious, or fraudulent statement may, in addition to other remedies available to the Government, subject me to civil penalties under the Program Fraud Civil Remedies Act of 1986 (45 CFR 79).	SIGNATURE OF PERSON NAMED IN 12c *(In ink. "Per" signature not acceptable.)*	DATE

DETAILED BUDGET FOR NEXT BUDGET PERIOD DIRECT COSTS ONLY	FROM	THROUGH	GRANT NUMBER
	15 July 1993	30 June 1994	HL44960-03

PERSONNEL (Applicant organization only) NAME	ROLE ON PROJECT	TYPE APPT. (months)	% EFFORT ON PROJ.	INST. BASE SALARY	DOLLAR AMOUNT REQUESTED (Omit cents) SALARY REQUESTED	FRINGE BENEFITS	TOTALS
Timothy M. Wick	Principal Investigator	12	20	64,690	12,938	3,520	16,458
James R. Eckman*	Co-Investigator	12	5		------	-----	------
Anjali Kumar		12	100	15,000	15,000		15,000
*Salary paid by Emory							
	SUBTOTALS →				27,938	3,520	31,458

CONSULTANT COSTS

None.

EQUIPMENT *(Itemize)*

None.

SUPPLIES *(Itemize by category)*

Tissue culture media, fetal bovine serum endothelial cell growth factors, adhesive proteins, buffers, antibiotics, EC mitogen	4,400	
Disposable plasticware (LabTek chambers, pipets, flasks, filters, gloves)	2,370	
Electrophoresis supplies, ELISA reagents	1,985	
Mcnoclonal antibodies, synthetic peptides	1,500	10,255

TRAVEL

To attend 2 scientific meetings	1,654

PATIENT CARE COSTS — INPATIENT / OUTPATIENT

ALTERATIONS AND RENOVATIONS *(Itemize by category)*

None.

OTHER EXPENSES *(Itemize by category)*

Machine shop, glass shop, electronics shop fees	250	
Publication fees, artwork, photography	853	1,103

SUBTOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD

CONSORTIUM/CONTRACTUAL COSTS

DIRECT COSTS $ _____

INDIRECT COSTS $ _____

TOTAL →

TOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD *(Enter on Page 1, Item 10a)* → $ 44,470

BUDGET JUSTIFICATION	GRANT NUMBER HL44960-03

SUPPLEMENTAL INFORMATION REGARDING *ITEMS* IN THE PROPOSED BUDGET FOR THE NEXT PERIOD WHICH REQUIRE EXPLANATION OR JUSTIFICATION. *(See instructions)*

Personnel: Fringe benefits are 27.2% of salary. Salary and fringe benefits for Dr. Wick have been reduced $20,670 to eliminate the funding overlap between this project and the Georgia Comprehensive Sickle Cell Center (see Other Support). All other costs reflect a 5% annual increase as funded in the original application.

Principal Investigator - Dr. Timothy M. Wick, Ph.D.: Funding is requested for the Principal Investigator to provide time to organize the study, coordinate *in vitro* investigations with clinical studies, perform experiments, analyze data, prepare manuscripts, hold regular laboratory meetings of the investigators, and develop progress reports. It is estimated that 50% of Dr Wick's time will be devoted to these tasks.

Graduate Student - Anjali Kumar: Ms. Kumar has been working in the laboratory since September 1991. She has recently begun doing sickle cell adherence studies and is responsible for most of the new data presented in the Results section. Ms. Kumar will devote 100% of her effort to this project. Ms. Kumar is (and will continue to be) responsible for the adhesion assays related to $\alpha 4\beta 1$/VCAM-1 mediated adherence, the red cell activation with phorbol ester, and the mechanism of thrombospondin-mediated adherence.

Supplies: Tissue culture costs are based upon current performance of 3 flow experiments per week as well as current usage and costs. Media, serum, growth factors, buffers, and other chemicals as well as plasticware, glassware, and gloves are required for cell cultures and adhesion assays. Monoclonal antibodies to adhesion receptors will be used to identify receptors that are necessary for sickle erythrocyte adhesion to endothelium.

Travel: Funds are requested for Dr. Wick to attend ASH and the annual Meeting of the Sickle Cell Disease Program to present research and interact with colleagues interested in similar and related areas of hematology and sickle cell anemia.

Other Expenses: Funds are requested to cover the cost photocopying and postage related to the transfer of data and data forms between Emory, Grady and Georgia Tech, medical illustrations and page costs. Machine shop charges are required to construct new adhesion systems.

CURRENT BUDGET PERIOD	FROM 1 July 1992	THROUGH 30 June 1993

The following pertains to your CURRENT PHS budget. This information may be used in determining the amount of support for the NEXT budget period.

A. CURRENT BUDGET	TOTAL ESTIMATED EXPENDITURES AND OBLIGATIONS (1)	ESTIMATED UNOBLIGATED BALANCE (2)	EXPLAIN ANY SIGNIFICANT ESTIMATED UNOBLIGATED BALANCE IN COLUMN 2 (3)
TOTAL DIRECT COSTS	$ 63,978	$0	
INDIRECT COSTS *(As provided)*	$ 39,346		
TOTALS →	$103,324		

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants listed on page 2. Begin with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	POSITION TITLE	BIRTHDATE *(Mo., Day, Yr.)*
Wick, Timothy M.	Assistant Professor	July 9, 1961

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of Colorado, Boulder CO	B.S.	1983	Chemical Engineering
Rice University, Houston, Texas	Ph.D.	1988	Chemical Engineering
Rice University, Houston, Texas	Post Doc	1988	Biochemistry and Chemical Engineering

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Professional Experience

9/88-present Assistant Professor, School of Chemical Engineering, Georgia Institute of Technology, Atlanta, GA
4/93-present Adjunct Assistant Professor, School of Mechanical Engineering, Georgia Tech, Atlanta, GA
2/88-9/88 Post-doctoral Research Associate, Department of Chemical Engineering, Rice University, Houston, TX
2/88-9/88 Post-doctoral Research Associate, Department of Chemical Engineering, Rice University, Houston, TX

Honors and Awards

1992 Lilly Foundation Teaching Fellowship
1991 Young Investigator Award Finalist. The 1991 World Congress on Medical Physics and Biomedical Engineering (Kyoto, Japan)
1991 American Heart Association-Georgia Affiliate, Grant-In-Aid
1991 The Whitaker Foundation, Biomedical Engineering Research Grant
1991 NIH-First Independent Research and Transition (FIRST) Award
1990, 91 Du Pont Young Faculty Award
1989 American Heart Association-Georgia Affiliate, Grant-In-Aid
1987 Beecham Award for outstanding original research presented at annual meeting of the Southern Society for Clinical Investigation, the Southern Section of the AFCR and the Southern Society for Pediatric Research
1986 Omega Chi Epsilon (National Chemical Engineering Honor Society)

Original Articles

1. Wick, T.M., J.L. Moake, M.M. Udden, S.G. Eskin, D.A. Sears and L.V. McIntire. "Unusually Large von Willebrand Factor Multimers Increase Adhesion of Sickle Erythrocytes to Endothelial Cells Under Controlled Flow." Journal of Clinical Investigation, **80**:905-910 (1987).
2. Wick, T.M., S.D. Doty and R.M. Nerem. "Influence of Fluid Mechanical Stresses on Vascular Cell Adhesion." In: Biomechanical Transport Processes, F. Mosora, C. Caro, C. Baquey, E. Krause, H. Schmid-Schönbein, C. Baquey, and R. Pelessier, eds, Plenum, New York, pp.283-292, 1991.
3. Wick, T.M. and V. Louis. "Cytoadherence of *Plasmodium falciparum*-Infected Erythrocytes to Human Umbilical Vein and Human Dermal Microvascular Endothelial Cells under Shear Conditions." Am J Tropical Med & Hygiene **45**: 578-586 (1991).
4. Swerlick, R.A., K. Lee, T.M. Wick, and T.J. Lawley. "Human Dermal Microvascular Endothelial but not Human Umbilical Vein Endothelial Cells Express CD36 *In Vivo* and *In Vitro*." Journal of Immunology, **148**:78-83 (1992).
5. Yoganathan A.P., T.M. Wick, & H. Reul. "The Influence of Flow Characteristics of Prosthetic Valves on Thrombus Formation." In: Thrombosis, Embolism, and Bleeding, E.G. Butchart and E. Bodnar, eds, ICR Publishers, London, pp. 123-148, 1992.
6. Wick, T.M. and V. Louis. "*Plasmodium fragile*: Cytoadherence of Parasitized Rhesus Monkey Erythrocytes to Human Endothelial Cells under Shear Flow Conditions." Experimental Parasitology, **74**:228-231 (1992).
7. Brittain, H.A., J.R. Eckman, and T.M. Wick. "Sickle Erythrocyte Adherence to Large Vessel and Microvascular Endothelium under Physiologic Flow is Qualitatively Different." The Journal of Laboratory and Clinical Medicine, **19**:538-545 (1992).
8. Wick, T.M., J.L. Moake, M.M. Udden, and L.V. McIntire. "Unusually Large von Willebrand Factor Multimers Preferentially Promote Young Sickle and Non-sickle Erythrocyte Adhesion to Endothelial Cells," Am J Hematology, **42**:284-292 (1993).

9. Johnson JK, RA Swerlick, P Millet, K Grady, TM Wick. "Cytoadherence of *Plasmodium falciparum*-Infected Erythrocytes to Microvascular Endothelium is Regulatable by Cytokines and Phorbol Ester," J Infect Dis, **167**:698-703 (1993).

¶10. Brittain, H.A., J.R. Eckman, R.J. Howard, and T.M. Wick. "Thrombospondin from Activated Platelets Promotes Sickle Erythrocyte Adherence to Human Microvascular Endothelium under Physiologic Flow: A Potential Role for Platelet Activation in Sickle Cell Vaso-occlusion," Blood, **81**:2137-2143 (1993).

11. Flaherty, A.L. and T.M. Wick. "Prolonged Contact with Blood Alters Surgical Gown Permeability," The American Journal of Infection Control, (**In press**) 1993.

12. Wick, T. M., J. K. Johnson, R. A. Swerlick, K. Grady, and P. Millet. "Cytokine Upregulation of CD36, but not ICAM-1, Increases Plasmodium falciparum-Infected Erythrocyte Adherence to Microvascular Endothelial Cells under Shear Conditions," In: Vascular Endothelium: Physiological Basis of Clinical Problems II, J. Catravas, A. Callow, N. Gillis, U. Ryan, A. Mantovani, and M. Yacoub, eds, Plenum, New York, (**In press**) 1993.

¶13. Wick, T.M., H.A. Brittain, R. Howard, and J.R. Eckman. "Thrombospondin from Activated Platelets Promotes Sickle Erythrocyte Adherence to Human Microvascular Endothelial Cells via CD36 and integrin receptors," In: Vascular Endothelium: Physiological Basis of Clinical Problems II, J. Catravas, A. Callow, N. Gillis, U. Ryan, A. Mantovani, and M. Yacoub, eds, Plenum, New York, (**In press**) 1993.

¶14. Swerlick, R.A., J.R. Eckman, A. Kumar, M. Jeitler, and T.M. Wick. "Reticulocytes from Patients with Sickle Cell Anemia Express the a_4/β_1 Integrin Complex and Bind to TNF-a Stimulated Endothelial Cells via a VCAM-1-a_4/β_1 Dependent Mechanism," Blood, **in review** (October 1992).

¶15. Gatewood, M., J.R. Eckman, and T.M. Wick. "Sickle Erythrocytes Stimulate Upregulation of Endothelial Cell VCAM-1, ELAM-1, and ICAM-1," (**In preparation**).

¶Indicates publications directly arising from currently funded research (HL44960)

Published Abstracts (selected)

1. Wick, T. M., L. V. McIntire, M. M. Udden, D. A. Sears, S. G. Eskin and J. L. Moake. "Unusually Large von Willebrand Factor Multimers Increase Adhesion of Sickle Erythrocytes to Endothelial Cells Under Controlled Flow," Clinical Research, **35**:603a,;1987.
2. Wick, T. M., J. L. Moake, M. M. Udden, and L. V. McIntire. "Unusually Large von Willebrand Factor Multimer Mediated Adhesion of Sickle and Young Normal Red Blood Cells to Endothelial Cells Can be Blocked by Antibodies to Glycoprotein Ib and IIb/IIIa," Blood, **70**:70a;1987.
3. Wick, T. M., L. V. McIntire, M. M. Udden, D. A. Sears, S. G. Eskin and J. L. Moake. "Unusually Large von Willebrand Factor Multimers Increase Adhesion of Sickle Erythrocytes to Endothelial Cells Under Controlled Flow," Clinical Research, **35**:16a:1987.
4. Wick, T. M., J. L. Moake, M. M. Udden and L. V. McIntire. "Similarities Between Young Non-Sickle Erythrocyte and Sickle Erythrocyte Adhesion to Human Endothelial Cells: Evidence that Young Red Cells Contain Receptors for von Willebrand Factor Multimers," Clinical Research, **36**:370a;1988.
5. Wick, T. M., J. L. Moake, M. M. Udden, and L. V. McIntire. "Unusually Large von Willebrand Factor Multimers Bind to Glycoprotein Ib-like and Integrin Receptors on Sickle and Young Non-Sickle Erythrocytes and Endothelial Cells: A Mechanism for Sickle and Other Young Erythrocyte Adhesion to Endothelial Cells," Blood, **72**:76a;1988.
6. Wick, T. M., H. A. Brittain, and J. R. Eckman. "Abnormal Adhesion of Sickle Erythrocytes to Human Microvascular Endothelial Cells is Due to Red Cell Factors and Collagen-Binding Plasma Proteins," FASEB Journal, **5**:A912;1991.
7. Flaherty, A., T. M. Wick, and J. R. Sommers. "Blood Permeability of Non-woven Surgical Gowns," Annals of Biomedical Engineering, **19**:605;1991.
8. Brittain, H. A., T. M. Wick, and J. R. Eckman. "Abnormal Adhesion of Sickle Red Blood Cells to Human Microvascular Endothelial Cells: A Potential Role for the Plasma Milieu in the Initiation of Vaso-occlusion," Annals of Biomedical Engineering, **19**:580:1991.
9. Wick, T. M., H. A. Brittain, and J. R. Eckman. "Adherence of Sickle Erythrocytes to Cultured Endothelial Cells Under Shear Flow: Influence of Endothelial Origin on the Mechanism of Adherence," 1991 Advances in Bioengineering, Winter Annual Meeting of the ASME, BED Vol. **20**:81-84;1991.
10. Wick, T. M., J. K. Johnson, R. A. Swerlick, K. Grady, and P. Millet. "Cytoadherence of *Plasmodium falciparum* Parasitized Red Blood Cells to Human Microvascular Endothelial Cell CD36 and ICAM-1 is Strain-Specific and Regulatable," FASEB Journal, **6**:1892A;1992.
11. Wick, T. M., J. K. Johnson, R. A. Swerlick, K. K. Grady, P. Millet. "Cytokine and Pharmacologic Regulation of *P. falciparum*-Infected Red Cell Adhesion to Microvascular Endothelial Cells under Shear Conditions," The American Journal of Tropical Medicine and Hygiene, **47**:149;1992.
12. Wick, T.M., H. A. Brittain, R. A. Swerlick, J. R. Eckman. "Thrombospondin from Activated Platelets Promotes Sickle Erythrocyte Adherence to Endothelium: A Potential Role for Platelet Activation in Sickle Cell Disease," Blood, **80**:76a;1992.
13. Wick, T. M., J. R. Eckman, A. Kumar, M. Jeitler, R. A. Swerlick. "Reticulocytes from Patients with Sickle Cell Anemia Express the $\alpha_4\beta_1$ Integrin Complex and Bind to TNF-α Activated Endothelial Cells via a VCAM-1/$\alpha_4\beta_1$ Dependent Mechanism," Blood, **80**:11a;1992.

BIOGRAPHICAL SKETCH

Give the following information for all new key personnel, consultants, and collaborators.
Copy this page for each person.

NAME	POSITION TITLE
James Robert Eckman	Associate Professor of Medicine

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of Minnesota,	B.A.	1965	Zoology
University of Minnesota	---	1966-67	MD/PhD Physiology
University of Minnesota	M.D.	1970	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individual who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

APPOINTMENTS:

Internship: Univ. of Minnesota Hospitals, Straight Medicine, 1970-1971
Residency: Univ. of Minnesota Hospitals, Internal Medicine, 1973-1973
Fellowship: Univ. of Minnesota Hospitals, Hematology 1974-1976
Instructor of Medicine, Univ. of Minnesota Medical School, 1973-1976
Chief Resident: Univ. of Minn. Hosp., 1973 Mpls. V. A. Hosp. 1974
Assistant Professor of Medicine, Univ. of Minn. Med. School, 1976-1978
Assistant Professor of Medicine, Emory Univ. School of Medicine, 1978-1980
Associate Professor of Medicine, Emory Univ. School of Medicine, 1980-
Assistant Professor of Pediatrics, Emory Univ. School of Medicine, 1984-

HONORS:

Phi Beta Kappa, Alpha Omega Alpha; Diplomate of the American Boards of Internal Medicine General Internal Medicine Boards, October 1974; Subspecialty of Hematology, June 1978.

ADVISORY COMMITTEES:

N.I.H. Behavioral Medicine Study Section, Member 1986, Chair 1988-1990; Georgia Advisory Council on Hemoglobinopathies, 1987-present;
Chairman, Sickle Cell Task Force of Georgia, 1982-1983;
Georgia Human Genetics Council, 1986-present;
Reviewer, HHS, Maternal and Child Health, Genetics, 1987, 1988, 1989, 1990; Council on Regional Genetics Networks, SERGG Sickle Cell Representative, 1988-present;
Council on Regional Genetics Networks, Chair, subcommittee on Quality Assurance for Hemoglobinopathy Screening, 1987-present;
Center for Disease Control Advisory Committee on Quality Assurance of Newborn Screening for Sickle Cell Disease, 1989.

ORIGINAL ARTICLES (Selected from a list 68 original articles and chapters):

1. J.R. Eckman, S. Modler, J.W. Eaton, E. Berger, and R.R. Engel: Host heme catabolism in drug sensitive and drug resistant malaria. J. Lab. Clin. Med. 90:767 - 770, 1977.
2. J.W. Eaton, J. R. Eckman, E. Berger, and H.S. Jacob: Erythrocyte oxidant sensitivity: Protection against severe malaria. Nature. 264:758-760, 1976.
3. J.R. Eckman and J.W. Eaton: Plasmodial gluthathione metabolism: Dependence upon the host cell. Nature. 278:754-756, 1979.
4. J.W. Eaton and J.R. Eckman: Malaria infection and host cell oxidant damage. In Biochemical and Clinical Aspects of oxygen. W.S. Caughey, ed. Academic Press, New York, 1979, pp 825-837.
5. N.L. Etkin, J.R. Mahoney, M.W. Forstoefel, J.R. Eckman, R.F. Gillum and J.W. Eaton. Racial differences in hypertension - associated red cell permeability. Nature. 297:588-589, 1982.
6. J.R. Eckman. Glutathione metabolism in malaria infected red cells. In Malaria and the Red Cell. J.W. Eaton and G. Brewer eds., Alan R. Liss, New York, pp

1-10. 1984.
7. L. Flores, I. Buchanan, D. Arnelle, V.M. Camp, M. Kutner, B.A. Faraj, J.R. Eckman, and A. Ragab. Pyridoxal-5'-phosphate levels in children with sickle cell disease. Amer. J. Pediatr. Hematol. Oncol. 10:236-240, 1988.
8. D.H. Barrett, I.E. Wisotzek, G.G. Abel, J.L. Rouleau, A.F. Platt, W.E. Pollard, and J.R. Eckman. Assessment of psychosocial functioning of sickle cell patients. Southern Med. J. 81:745-750, 1988.
9. M. Allon, L. Lawson, J.R. Eckman, V. Delaney, and Bourke, E. The effects of nonsteroidal antiinflammatory drugs on renal function in sickle cell anemia. Kidney Int. 34:500-506, 1988.
10. A.R. Bishop, J. Roberson, J.R. Eckman, and L.L. Fleming. Total hip arthroplasty in patients who have sickle hemoglobinopathy. J. Bone and Joint Surg 70-A: 853-855, 1988.
11. J.R. Eckman. Sickle cell anemia: Pathophysiology and preventive treatment. The Emory Univ. J. Med. 2:140-146, 1988.
12. A.P. Platt and J.R. Eckman. Management of pain in sickle cell anemia patients. J. Amer. Acad. Phys. Assist. 2:104-113, 1989.
13. M.S. Harris and J.R. Eckman. Georgia's experience with newborn screening - 1981 - 1985. Pediatrics 83:858-860,1989.
14. J.R. Eckman, T.R. Kinney, and M.S. Harris. Newborn for hemoglobinopathies: Facilitation by a TASCS Force. Annals New York Acad. Sci. 565:376-378, 1989.
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OTHER SUPPORT *(Use continuation pages if necessary)*	GRANT NUMBER HL44960-03

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Name Timothy M. Wick Active X Pending _____ None _____

a. Source and identifying no. The Whitaker Foundation P.I. T.M. Wick

Title Endothelial Cell Activation and Blood Cell Adhesion in Atherosclerosis

b. Your role on project Principal Investigator % Effort 25%

c. Dates and costs of entire project 1 July 1991 - 30 June 1994 ($179,999)

d. Dates and costs of current year 1 July 1992 - 30 June 1993 ($48,444 direct)

e. Specific aims of project To elucidate the mechanism of adherence of monocytes to endothelial cells under physiologically relevant conditions.

f. Describe scientific and budgetary overlap No scientific or budgetary overlap.

g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)

None.

Timothy M. Wick Active (continued)

2.

a. Source and Identifying Number: NIH 1-PO1-HL48482-01
P.I. James R. Eckman, M.D., Associate Professor of Medicine in Hematology/Oncology, Assistant Professor of Pediatrics in Human Genetics, Emory University School of Medicine, Atlanta, Georgia.

Title: Georgia Comprehensive Sickle Cell Center

b. Your role on project: Collaborating Investigator **% Effort:** 30%

c. Dates and costs of entire project: 1 April 1993 - 31 March 1998 $5,085,570 (direct)

d. Dates and costs current year: 1 April 1993 - 31 March 1994 $867,809 (direct)

e. Specific aims of project: The primary goal of the Georgia Comprehensive Sickle Center is to provide basic and clinical research, education, laboratory diagnosis, counseling and patient care in sickle cell syndromes. Research projects will address issues of pathophysiology and treatment for important complications in patients with these disorders. Dr. Wick's role in the Center is to develop and execute investigations into the effects of sickle red blood cells on endothelial cell morphology and function. These important studies will provide insight into the mechanism of sickle red cell induced endothelial cell damage and the related clinical complications (such as stroke), not necessarily related to microvascular occlusion. We are testing the hypothesis that sickle erythrocytes alter endothelial cell morphology, metabolic processes, and function. Specifically, we have data that sickle red blood cells inhibit endothelial cell responses to arterial levels of shear stress, sickle cells induce endothelial cell adhesion molecule expression, and stimulation of endothelial cells with sickle red cells increases the affinity of the endothelium for sickle erythrocytes. The SCORE research that involves Dr. Wick is complementary to the erythrocyte adherence studies related to microvascular occlusion ongoing under the current R29 award.

f. Describe budgetary and scientific overlap: The budgetary overlap is limited to the PI's salary.

g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.): The PI's salary on this project will be reduced from 50% to 20%. This reduction eliminates the funding overlap between the R29 and Dr. Wick's funding from the SCORE which arose because the projects are complementary, and some of Dr. Wick's efforts benefit both projects simultaneously. For example, study design, sample collection, data analysis, research group meetings with collaborators, report writing, etc. for the projects are interrelated and Dr. Wick, as PI, actively participates in each of these areas. In addition, Dr. Wick is heavily involved in the design and execution of experiments related to both projects. The 20% effort on the R29 and the 30% effort on the SCORE and the related revised budgets accurately reflect Dr. Wick's commitment to and participation in both projects. Note that with these budget revisions, Dr. Wick still devotes 50% effort to his studies in the area of sickle cell/endothelial adherence and the related effects of sickle cells on endothelial cell biology. With this revision, the PI's salary and fringe benefits have been reduced by $20,670 in the R29 for years 3-5.

The aims will be adjusted slightly as indicated in the report (see **Plans**). This adjustment is made solely on the basis of our results to date as reported. We will focus our efforts on identifying the adherence mechanism(s) utilized when sickle cells are suspended in autologous plasma. This emphasis is based on the knowledge that plasma is the milieu *in vivo*. and our belief that integrin receptors account for a significant fraction of the plasma-mediated adherence. We are interested in investigating anti-integrin receptor peptides for their ability to inhibit or reverse adherence in a plasma environment in order to provide data of use in the development of anti-adhesion therapies.

With this budget adjustment, NIH will be funding 1 graduate student and Dr. Wick for 20% effort on the R29 and 2 graduate students and Dr. Wick for 30% effort on the SCORE research. Clearly our preliminary data as detailed in the proposals justify this level of funding for sickle cell/endothelial cell interactions.

3.
a. Source and Identifying Number: NIH 1T32GM08433-02 **P.I.** Robert M. Nerem, Ph.D.

Title: Cellular Engineering Training Program

b. Your role on project: Collaborating Faculty **% Effort:** 5%

c. Dates and costs of entire project: $339,208 (26 September 1991 - 31 August 1995)

d. Dates and costs current year: $59,460 (1 July 1992 - 30 June 1993)

e. Specific aims of project: This project provides funds for predoctoral students studying Cellular Engineering.

f. Describe budgetary and scientific overlap: None.

g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.): None.

4.
Source and Identifying Number: The Whitaker Foundation **P.I.** Robert M. Nerem, Ph.D.

Title: Biomedical Engineering Education: An Interdisciplinary Tissue Engineering Education and Research Program

b. Your role on project: Participating faculty **% Effort:** 0%

c. Dates and costs of entire project: $3,000,000 (1 September 1993 - 31 August 1996)

d. Dates and costs current year: $1,500,000 (1 September 1993 - 31 August 1994)

e. Specific aims of project: This grant provides funds for laboratory space renovation, the hiring of six new faculty in Tissue Engineering, and a limited number of graduate student stipends.

f. Describe budgetary and scientific overlap: None.

g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.): None.

PENDING

None.

PLANNED

None.

CONTINUATION PAGE. START WITHIN MARGINS INDICATED

5.
Source and Identifying Number: NSF BCS9111761 **P.I.** Robert M. Nerem, Ph.D.

Title: Reconstitution of a blood vessel in culture

b. Your role on project: Co-investigator **% Effort:** 5%

c. Dates and costs of entire project: $443,740 (1 September 1991 - 28 February 1995)

d. Dates and costs current year: $178,461 (1 September 1992 - 31 August 1993)> ($10,000 annual direct costs for Dr. Wick)

e. Specific aims of project: Dr. Wick will evaluate the thrombogenicity of tissue engineered blood vessels developed in Dr. Nerem's lab.

f. Describe budgetary and scientific overlap: None.

g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.): None.

OTHER SUPPORT *(Use continuation pages if necessary)*	GRANT NUMBER HL44960-03

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Name James R. Eckman Active X Pending _____ None _____

a. Source and identifying no. NIH/NHLBI, 1P60 HL48482-01 P.I. James Eckman

Title Georgia Comprehensive Sickle Cell Center

b. Your role on project Principal Investigator % Effort 32%

c. Dates and costs of entire project 4/1/93 - 3/31/98: $8,299,001

d. Dates and costs of current year 4/1/93 - 3/31/94: $1,237,856

e. Specific aims of project The primary goal of the grant is to provide basic and clinical research, education, laboratory diagnosis, counseling and patient care in sickle cell syndromes. Research projects will address issues of pathophysiology and treatment for important complications in patients with these disorders.

f. Describe scientific and budgetary overlap None.

g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)

None.

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Name James R. Eckman Active X Pending _____ None _____

a. Source and identifying no. Georgia D.H.R., 93-50288 P.I. Fulton-Dekalb Hosp. Authority

Title Treatment of Sickle Cell Anemia

b. Your role on project Supervision of clinical care % Effort 70%

c. Dates and costs of entire project 7/1/86 - 6/30/93: $605,000 per year

d. Dates and costs of current year 7/1/92 - 6/30/93: $605,000

e. Specific aims of project Provision of clinical care of sickle cell patients.

f. Describe scientific and budgetary overlap None.

g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)

None.

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Name James R. Eckman Active X Pending _____ None _____

a. Source and identifying no. H.H.S. Block Grant P.I. Fulton-Dekalb Hosp. Authority

Title Cord Blood Screening for Hemoglobinopathies

b. Your role on project Supervision of newborn screening program % Effort 5%

c. Dates and costs of entire project 10/1/85 - 9/30/93: $58,156 per year

d. Dates and costs of current year 10/1/92 - 9/30/93: $58,156

e. Specific aims of project To provide newborn screening for sickle cell disease and other hemoglobinopthies for all newborn babies at Grady Memorial Hospital.

f. Describe scientific and budgetary overlap None.

g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)

None.

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Name James R. Eckman Active X Pending _____ None _____

a. Source and identifying no. NIH/NHLBI, 1U01 45692-01 P.I. Samuel Charache

Title Hydroxyurea in Sickle Cell: Subcontract to Johns Hopkins

b. Your role on project Local Principal Investigator % Effort 5%

c. Dates and costs of entire project 5/1/91 - 4/30/96: $346,864 per year entire project

d. Dates and costs of current year 5/1/92 - 4/30/93: $67,370

e. Specific aims of project This is a multi-center study of hydroxyurea in sickle cell anemia to determine whether or not treatment with hydroxyurea titrated to maximum tolerated doses will reduce to at least 50% the frequency of vaso-occlusive (painful) crisis. The secondary objectives are to establish the relationship of fetal hemoglobin levels and other patients or treatment characteristics to the occurrence of vaso-occlusive (painful) crises, and the effect of treatment on the quality of patients' lives.

f. Describe scientific and budgetary overlap None.

g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)

None.

OTHER SUPPORT

(Use continuation pages if necessary)

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Name James R. Eckman Active X Pending _____ None _____

a. Source and identifying no. National Cancer Institute P.I. Melvin Moore

Title Grady Memorial Hospital Clinical Oncology Program

b. Your role on project Co-Investigator % Effort 5%

c. Dates and costs of entire project 7/1/90 - 6/30/93: $24,959 per year

d. Dates and costs of current year 7/1/92 - 6/30/93: $24,959

e. Specific aims of project To enroll minority patients in cancer chemotherapy trials.

f. Describe scientific and budgetary overlap None.

g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)

Effort is to be reduced to 0%.

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Name James R. Eckman Active _____ Pending _____ X None _____

a. Source and identifying no. Georgia D.H.R. P.I. Fulton-Dekalb Hosp. Authority

Title Treatment of Sickle Cell Anemia

b. Your role on project Supervision of clinical care % Effort 50%

c. Dates and costs of entire project 7/1/93 - 6/30/94: $575,000 per year

d. Dates and costs of current year 7/1/93 - 7/30/94: $575,000

e. Specific aims of project Provision of clinical care of sickle cell patients.

f. Describe scientific and budgetary overlap None.

g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)

Dr. Eckman's previously proposed clinical effort and salary on this grant will be reduced to 50% because of the greater effort devoted to research.

OTHER SUPPORT

(Use continuation pages if necessary)

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Name James R. Eckman Active _____ Pending X None _____

a. Source and identifying no. H.H.S. Block Grant P.I. Fulton-Dekalb Hosp. Authority

Title Cord Blood Screening for Hemoglobinopathies

b. Your role on project Supervision of newborn screening program % Effort 5%

c. Dates and costs of entire project 10/1/93 - 9/30/94: $58,156 per year

d. Dates and costs of current year 10/1/93 - 9/30/94: $58,156

e. Specific aims of project To provide newborn screening for sickle cell disease and other hemoglobinopathies for all newborn babies at Grady Memorial Hospital.

f. Describe scientific and budgetary overlap None.

g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)

None.

1. Specific Aims

The tendency for hemoglobin SS to polymerize at low oxygen tension is assumed to be the dominant factor in sickle cell pathology. Since morphological sickling is delayed after hemoglobin deoxygenation, factors which delay red cell microcirculatory transit are likely antecedents to microvascular occlusion, ischemic tissue damage, and pain episodes characteristic of sickle cell anemia. Our central hypothesis is that sickle erythrocyte adherence to microvascular endothelium delays erythrocyte microcirculatory transit. This partial obstruction allows for intracapillary red cell sickling leading to complete occlusion (1). Our data clearly indicate that plasma, red cell and endothelial cell factors as well as local hemodynamics all likely contribute to adherence and occlusion *in vivo* (2-7). Our **specific aims for this project** are to (i) characterize differences in sickle red blood cell adherence to phenotypically diverse endothelium (veins, arteries, microvessels); (ii) identify specific plasma factors, red cell membrane abnormalities, and endothelial ligands which promote sickle red blood cell (SRBC) adherence, and (iii) characterize the interpatient and intrapatient adherence mechanisms and degree of adherence during asymptomatic periods and pain episodes. The long-term goal of this research is to discover the mechanisms and extent of sickle red cell adherence to different vascular sites during pain episodes and asymptomatic periods. These studies will be invaluable to the development of effective anti-adhesion therapies to eliminate or reduce the ischemic tissue damage associated with blood vessel occlusion in sickle cell anemia.

2. Studies and Results

Methods

Adherence of sickle red blood cells (SRBC) to cultured human umbilical vein (HUVEC) and microvascular (MEC) endothelial cells was quantified under dynamic flow conditions *in vitro* essentially as described in the original grant application (6).

Thrombospondin-Mediated Sickle Red Cell Adherence to Microvascular Endothelium

We have previously reported that thrombospondin (TSP), possibly released from activated sickle platelets *in vivo*, promotes sickle red cell adherence to MEC under physiological flow conditions (2). A main goal for this budget period was further elucidation of the mechanism of TSP-mediated adherence. TSP binds to CD36 and the vitronectin receptor on MEC (2). However, it is not known whether TSP receptors are expressed on SRBC. We (Figure 1 & ref. 3) and others (8) have recently demonstrated that sickle reticulocytes express CD36, a TSP receptor (9). Normal reticulocytes also express CD36, but to a much lesser degree (data not shown - see reference 25). As shown in Figure 2, preincubation of SRBC with anti-CD36 antibody quantitatively blocks TSP-mediated SRBC adherence to MEC.

When either SRBC or MEC are incubated with TSP, TSP promotes SRBC adherence (Fig 3). However separate incubation of both the SRBC and MEC with TSP prior to a flow adhesion assay does not promosimultaneous occupation of TSP receptors on both the SRBC and MEC inhibits adherence.

We have previously reported that antibodies to either the vitronectin receptor ($\alpha_V\beta_3$) or CD36 quantitatively inhibit TSP-mediated SRBC adherence to MEC (2). Considerable ongoing debate does not clarify whether both of these receptors are TSP receptors or whether CD36 and $\alpha_V\beta_3$ are closely opposed such that antibody occupation of one receptor sterically inhibits TSP access to the other receptor (10-12). To address this issue, we have utilized the sextapeptide (CSVTCG) sequence that is the purported CD36-binding domain of TSP (13) and the RGD sequence that binds to integrin receptors (14) to investigate further the role of CD36 and VnR in TSP-mediated adherence.

Preincubation of either SRBC or MEC with CSVTCG also abolishes TSP-mediated adherence (Fig 4). Similarly, preincubation of MEC with RGD abolished adherence (Fig 5).

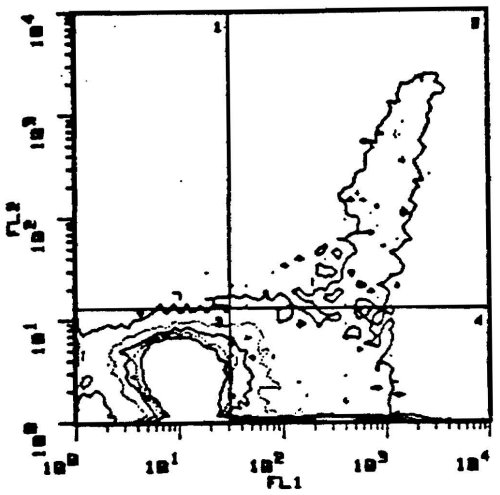

Fig 1: Sickle RBC express CD36, a TSP receptor. SRBC were labeled with both thiazole orange (FL1) and anti-CD36 antibody (FL2) and analyzed by fluorescence activated cell sorting (FACS) (24). The population of RBC in quadrant 2 represent 8% of the patients RBC that are CD36-expressing reticulocytes.

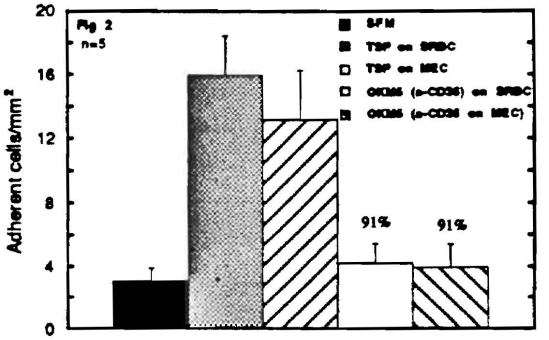

Fig 2: Anti-CD36 antibody inhibits TSP-mediated adherence to MEC. Preincubation of either SRBC or MEC with OKM5 antibody inhibits SRBC adherence to a similar extent (shown in parentheses). Unbound antibody was washed away prior to the adherence assay. Data are mean±SEM for the number of experiments in this and all plots (except where indicated).

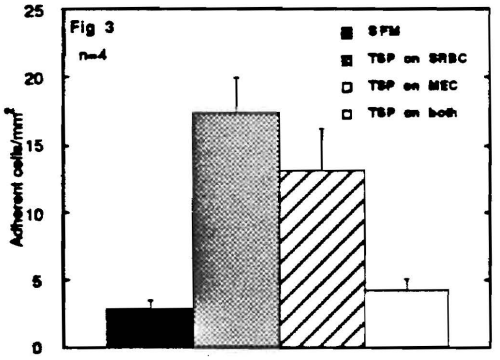

Fig 3: TSP mediates adhesion of SRBC to TSP. Preincubation of either SRBC or MEC with TSP promotes SRBC adherence; incubation of both MEC and SRBC with TSP does not promote adherence.

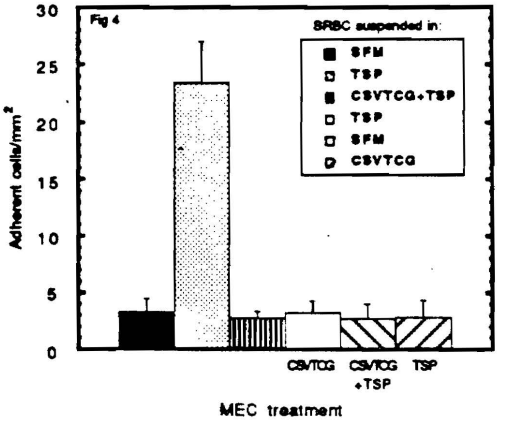

Fig 4: TSP-mediated SRBC adherence is blocked by the antagonist peptide CSVTCG. Incubation of either MEC of SRBC with CSVTCG peptide quantitatively inhibits SRBC adherence to MEC.

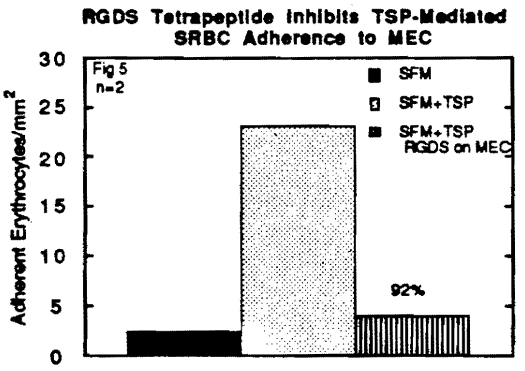

Fig 5: Preincubation of MEC with RGDS peptide inhibits TSP-mediated SRBC adhesion to MEC 92%.

Endothelial Stimulation

Using monoclonal antibodies and fluorescence activates cell sorting (FACS) we have recently demonstrated that a subpopulation of young reticulocytes in sickle blood express the $\alpha_4\beta_1$ (VLA-4) integrin receptor (Fig 6). Normal reticulocytes also express VLA-4, but to a lesser degree (data not shown). Sickle reticulocytes do not express the α_1, α_2, α_3, α_5, α_6, α_v, β_2, β_3 integrin receptors (Fig 6). The ligand for $\alpha_4\beta_1$ is VCAM-1 (15) which is expressed by HUVEC (16) and MEC (17) after stimulation with cytokines such as tumor necrosis factor (16,17). SRBC, but not NRBC, adhere to TNF-α stimulated HUVEC (Fig 7) and this adherence is inhibited greater than 70% by incubating SRBC with an anti-$\alpha_4\beta_1$ antibody or the HUVEC with an anti-VCAM-1 antibody (Fig 8). TNF-α also induces VCAM-1 expression on MEC (17). In one experiment, SRBC also adhered to cytokine activated MEC via a $\alpha_4\beta_1$/VCAM-1 dependent adherence pathway (Fig 9) similar to that observed for HUVEC.

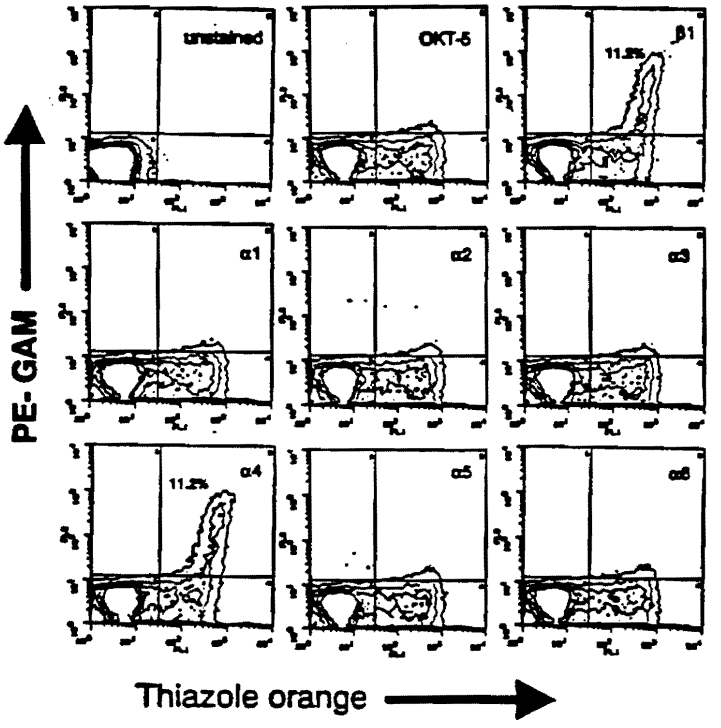

Fig 6: SRBC were labeled with thiazole orange (to identify reticulocytes) (FL1) and an anti-α_4 or anti-β_1 (FL2) antibody and analyzed by FACS as described in Fig 1. The cells stained positive for both α_4 and β_1.

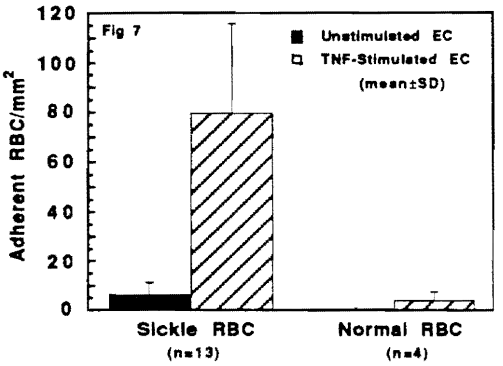

Fig 7: TNF-a stimulated HUVEC support SRBC Adherence. Sickle, but not normal ,RBC adhere to TNF-α stimulated HUVEC.

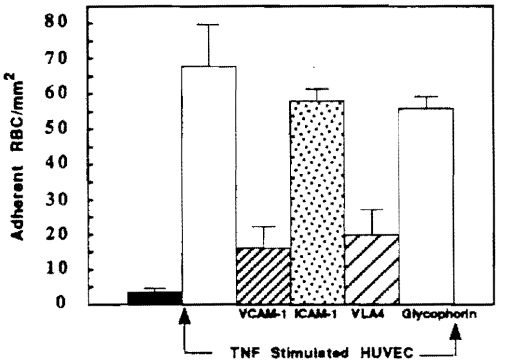

Fig 8: SRBC adhere to cytokine stimulated HUVEC via a VLA-4/VCAM-1 dependent mechanism. Anti-VCAM-1 or anti-VLA-4 antibody inhibited SRBC adherence to TNF-α stimulated HUVEC by 80% and 74%, respectively. Neither incubation of HUVEC with anti-ICAM-1 or SRBC with anti-glycophorin inhibited SRBC adherence.

Sickle Erythrocyte Stimulation

VLA-4 is constitutively expressed on a subpopulation of sickle reticulocytes (Fig 6) and promotes adherence to activated HUVEC via VCAM-1 (Fig 7). Recent data suggest that β_1 integrins, including VLA-4, can be stimulated to exhibit higher affinity for their ligands (e.g. activated) (18) by phorbol esters and other agonists. In order to test the hypothesis that erythrocyte activation elevates sickle cell adherence to endothelium, we stimulated SRBC with phorbol dibutyrate (PDBu) prior to the flow adherence assays. As seen in Figure 10, PDBu activated sickle, but not normal, RBC are more adherent to cultured endothelium as compared to unstimulated SRBC. These adherence assays were conducted **in the absence of any adherence proteins** and without endothelial stimulation. Thus, the phorbol ester induced SRBC adherence appears to be via a pathway not dependent upon exogenous proteins or endothelial cell activation.

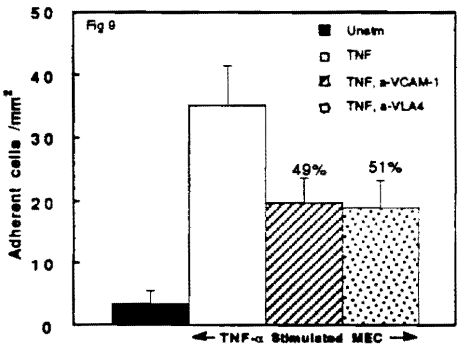

Fig 9: Sickle RBC adhere to TNF-α stimulated MEC. MEC were activated with TNF, washed SRBC suspended in SFM (containing no adhesive proteins) were perfused over the monolayer and adherent sickle cells were enumerated as described in Figs 7&8. Adherence was partially blocked by anti-$\alpha 4\beta_1$ antibody on SRBC or by anti-VCAM-1 antibody on the MEC.

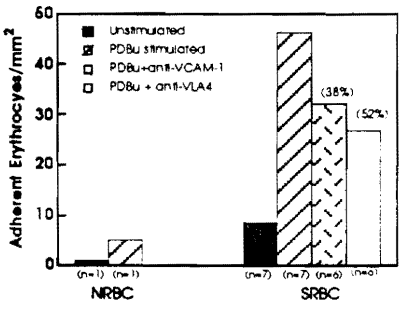

Fig 10: Phorbol ester stimulation of SRBC elevates SRBC adherence to HUVEC. SRBC suspended in SFM were pretreated with phorbol ester (PDBu) and adherence to unstimulated HUVEC was quantified. In some experiments, HUVEC were pretreated with anti-VCAM-1 or SRBC were pretreated with anti-VLA-4. The percent antibody inhibition is shown above each bar.

3. Significance

Effect of Endothelial Cell Phenotype

We have recently reported that SRBC adherence to microvas19). Notably, high molecular weight von Willebrand factor multimers promote adherence to HUVEC but not MEC (19). In contrast, autologous plasma promotes very high SRBC adherence to MEC, but only low levels of SRBC adherence to HUVEC (19). The MEC are likely predominately of capillary origin, whereas the HUVEC may closely mimic the post-capillary venule endothelium (19). Thus, these data indicate that multiple and different pathways exist for SRBC adherence - and that (for example) plasma-mediated adherence in the capillaries and adherence to post-capillary venule endothelium via high molecular weight vWF possibly act in tandem to provide greater blood flow impediment in the microcirculation.

Here we report of two additional adherence pathways; one mediated by TSP and its receptors on SRBC (CD36) and microvascular endothelium (CD36 and $\alpha_v\beta_3$) and the other mediated by SRBC VLA-4 ($\alpha_4\beta_1$) binding to VCAM-1 expressed on TNF-α stimulated endothelium. Recently, Sugihara, *et al.* (8) reported that TSP also mediates SRBC adherence to HUVEC via CD36 on the SRBC and unidentified receptor(s) on HUVEC. However, an RGDS peptide inhibited TSP-mediated binding (8) indicating that HUVEC integrin receptors are involved in adherence. Thus, the mechanism of TSP mediated SRBC adherence to MEC and HUVEC is apparently analogous. Similarly, the $\alpha_4\beta_1$/VCAM-1 adherence is significant for both TNF-α stimulated HUVEC (Fig 8) and MEC (Fig 9). Thus, these two pathways supports adherence of SRBC to both large vessel and microvascular endothelium and may participate in adherence *in vivo* in both the capillaries and post-capillary venules.

Endothelial Cell Activation

One of our most significant observations is that SRBC express the $\alpha_4\beta_1$ integrin receptor and adhere to TNF-α activated HUVEC and MEC. Cytokine levels are elevated in sickle patients during asymptomatic periods and during acute illness (20). It is possible, especially in light of our recent data (Figs 6-9), that cytokine production may induce endothelial cell VCAM-1 expression and SRBC adherence *in vivo*, leading to microvascular occlusion. These data suggest that anti-cytokine therapy, to inhibit VCAM-1 expression, may be an alternative therapeutic strategy to prevent, minimize, or reverse sickle cell adherence to vascular endothelium and the accompanying necrotic tissue damage. We will further explore this hypothesis by monitoring patient cytokine levels in order to attempt correlations between plasma cytokine level, *in vitro* endothelial adherence, and disease severity.

Sickle Cell Activation

The data of Figure 10 suggest that sickle red blood cells can exhibit higher affinity for endothelial cell receptors when stimulated with agonists such as phorbol ester. These data suggest an intriguing hypothesis that the SRBC can become 'activated' *in vivo*, exhibit greater affinity for the endothelium, and lead to increased endothelial adherence and vaso-occlusion **in the absence of changes in plasma concentrations of adhesive proteins or endothelial cell activation**. Since these data are only preliminary, we do not know if the increased adherence is due solely to $\alpha_4\beta_1$ 'activation' as we originally hypothesized or if other receptors are involved under these stimulation conditions. However, it is unlikely that PDBu is exerting its effect through endothelial cell activation since the phorbol ester is incubated with the red cells and the red cells are washed prior to the adherence assay. Obviously, we will continue to explore this hypothesis and preliminary data to further determine whether an 'activation' state exists for SRBC.

4. Plans

In general, we do not anticipate significant deviation from the original proposal. However, the future work will be focused on the data of most promise in elucidation of the mechanisms of sickle red cell/endothelial cell adherence *in vivo*, in order to provide data that is of use to developing anti-adhesion therapies. We will focus on identifying the plasma components responsible for sickle cell adherence and will identify which adhesion pathway or pathways are most prominent in the plasma milieu. Thus, we will investigate adherence when sickle red cells are suspended in autologous plasma

(as opposed to incubated with purified proteins) since this mimics the *in vivo* milieu. Also, we will continue to identify and characterize the sickle red cell membrane receptor(s) and their ligands on endothelial cells.

The Role of Integrin Receptors in Plasma-Mediated SRBC Adherence

We have characterized several adherence pathways involving integrin receptors, including: VCAM-1/$\alpha_4\beta_1$ (Fig 8), TSP/$\alpha_v\beta_3$ (ref 2), high molecular weight vWF/integrin receptor (ref 7). Others have shown that fibrinogen (21) and fibronectin (4) also promote SRBC, possibly through endothelial integrin receptors (22). We hypothesize that adherence to integrin receptors (possibly mediated by a variety of proteins) accounts for a significant fraction of the adherence *in vivo*. We will test this hypothesis by examining SRBC adherence when erythrocytes are suspended in autologous plasma. We will identify specific plasma factors which promote adherence, especially integrin receptor agonist proteins (e.g. TSP, vWF, fibrinogen, fibronectin). In parallel, we will investigate peptides based on the RGD motif (which competes with RGD-containing proteins for integrin receptors [23]) for their ability to inhibit plasma-mediated adherence. In general, these peptides are commercially available. These experiments will be of interest because the experimental system we utilize closely mimics the microvascular milieu *in vivo*. That is, our SRBC adhesion experiments will be performed in autologous plasma on human microvascular endothelium under shear conditions typical of that in the microcirculation. Successful identification of RGD-containing peptides that inhibit plasma-mediated adherence could form the basis for anti-adhesion therapies for patients.

"Activated" Sickle Red Cells

The data of Figure 9 indicate that sickle red cell affinity for endothelial cell ligands can be significantly increased after stimulation with phorbol ester. This suggests that the red cell may periodically exhibit greater adhesivity for the endothelium *in vivo*. We plan to further explore this hypothesis with the following experiments. The fact that VLA4 on the SRBC accounts for only approximately half of the adherence when SRBC are stimulated with PDBu, suggests that other endothelial receptors are involved. Thus, using our monoclonal antibodies to a variety of adhesion receptors (VCAM-1, ELAM-1, ICAM-1, CD36, GPIb, $\alpha_v\beta_3$, etc.) we will determine whether the stimulated RBC adhere via a known SRBC adherence pathway. In addition, since we have not directly demonstrated that the $\alpha_4\beta_1$ positive RBC are the adherent RBC in response to phorbol ester we will test $\alpha_4\beta_1$ positive and $\alpha_4\beta_1$ negative cells subfractions of RBC to establish whether $\alpha_4\beta_1$ on SRBC is involved in adherence. These subfractions will be generated by density gradient centrifugation (25) (since most of the $\alpha_4\beta_1$ positive cells are reticulocytes which have low density) or fluorescence activated cell sorting (24). We have utilized both of these technologies and do not anticipate any difficulty.

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19. Brittain HA, JR Eckman, and TM Wick. "Sickle erythrocyte adherence to large vessel and microvascular endothelium under physiologic flow is qualitatively different." J Lab Clin Med 19:538; 1992.
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5. Human Subjects

a. General Guidelines

i. Proposed Use

Patients with sickle-cell syndromes (HbSS, HbSC, HbS β-thalassemia) not receiving anticoagulant therapy and without evidence of pregnancy, obvious infection, thromboembolic disease or liver disease will be eligible for this study. Patients will be studied once in pain crisis and twice during asymptomatic periods. An age and sex matched population of normal black individuals will serve as a control population. Approximately twenty patients and twenty control subjects, aged eighteen or older, will be studied annually. Ten milliliters of blood will be drawn by venipuncture for each experiment.

ii. Specimen Usage

None of the data from the experiments will be used for diagnosis or treatment of specific individuals.

iii. Patient Recruitment

Patients from the Sickle Cell Center or the in-patient service at Grady Memorial Hospital, Atlanta, GA and hospital staff will be recruited by Dr. James R. Eckman. Subjects will agree to participate in this study by signing a consent form approved by Georgia Tech and Emory University School of Medicine IRBs. The consent form explains the nature of the study, the details of blood collection, risks associated with drawing blood, the availability of personnel to discuss the results of the study, the assurance of anonymity, and the ability to withdraw from the study at any time without penalty or loss of benefits.

iv Potential Risks

The risks of drawing blood are minimal and include slight pain, bruising, and infection at the site of puncture. No viable alternative for drawing human blood exists.

v. Procedures to Minimize Risk

Patient confidentiality will be ensured by assigning a code to each patient studied (SS1, AA1 for sickle and normal donor, respectively) to be used when all data is reported. Blood will be drawn at Grady Hospital under the supervision of Dr. James R. Eckman, director of the Sickle Cell Clinic. Dr. Eckman will be available to answer questions and to arrange for emergency medical care if a medical problem develops during the course of this study.

vi. Justification

The risk of blood drawing is minimal compared to potential benefits of a better understanding of clotting abnormalities in sickle cell syndromes and their relationship to pain crisis.

b. Gender and Minority Inclusions

Study subjects will be patients diagnosed with sickle cell syndromes as defined above. These patients will primarily be of African descent, however no patients will be included or excluded on the basis of race. The study population will consist of approximately equal numbers of men and women. Exclusion criteria will be solely based on medical criteria as described above. Control subjects (volunteers without hemoglobinopathies) will be age, sex, and race-matched. These volunteers are recruited from the hospital staff at Grady Memorial Hospital in Atlanta.

6. Vertebrate Animals

None.

7. Publications (from this project)

a. Journal Articles

Brittain HA, JR Eckman, and TM Wick. Sickle erythrocyte adherence to large vessel and microvascular endothelium under physiologic flow is qualitatively different. The Journal of Laboratory and Clinical Medicine, 19:538-545 (1992).

Brittain HA, JR Eckman, RJ Howard TM Wick: Thrombospondin from activated platelets promotes sickle erythrocyte adherence to human microvascular endothelium under physiologic flow: A potential role for platelet activation in sickle cell vaso-occlusion. Blood, 81:2137-2143 (1993).

Swerlick RA , JR Eckman, A Kumar, M Jeitler, and TM Wick. Reticulocytes from patients with sickle cell anemia express the a4/ß1 integrin complex and bind to TNF-α stimulated endothelial cells via a VCAM-1-a4/ß1 dependent mechanism," Blood, **in review** (October 1992).

b. Abstracts and Meeting Presentations

Wick, TM, HA Brittain, RA Swerlick, JR Eckman. "Thrombospondin from Activated Platelets Promotes Sickle Erythrocyte Adherence to Endothelium: A Potential Role for Platelet Activation in Sickle Cell Disease," Blood, **80**:76a;1992.

Wick, TM, JR Eckman, A Kumar, M Jeitler, RA Swerlick. Reticulocytes from patients with sickle cell anemia express the $\alpha 4\beta 1$ integrin complex and bind to TNF-α activated endothelial cells via a VCAM-1/$\alpha 4\beta 1$ dependent mechanism," Blood, **80**:11a;1992.

Wick TM, HA Brittain, R Howard, and JR Eckman. "Thrombospondin from Activated Platelets Promotes Sickle Erythrocyte Adherence to Human Microvascular Endothelial Cells via CD36 and integrin receptors," NATO Advanced Studies Institute "Vascular Endothelium: Physiological Basis of Clinical Problems II," Rhodes, Greece (June 1992).

Wick, TM and JR Eckman. "Sickle Erythrocyte Adherence to Endothelium: The Role of Endothelial Phenotype and Acute Phase Reactants," NIH Investigator's Meeting on Sickle Cell Disease, Bethesda, MD (September 1992).

Wick TM, HA Brittain, JR Eckman. "Thrombospondin from Activated Platelets Mediates Sickle Red Cell Adhesion to Human Microvascular Endothelium. 1992 Annual Fall Meeting of the Biomedical Engineering Society, Salt Lake City, UT (October 1992).

Brittain HA, TM Wick and JR Eckman. "Thrombospondin from Activated Platelets Mediates Sickle Red Cell Adhesion to Human Microvascular Endothelium: A Potential Role for Intravascular Coagulation in Sickle Cell Anemia," 1992 Annual Meeting of the American Institute of Chemical Engineers, Miami, FL (November 1992).

Wick TM and J R Eckman. "Multiple and Different Mechanisms of Sickle Erythrocyte Adherence to Large Vessel and Microvascular Endothelium Under Flow Conditions: Pathophysiological Implications," 1993 ASME Summer Bioengineering Conference, Breckenridge, CO (June 1993).

Wick TM, A Kumar, JR Eckman, and RA Swerlick. "Sickle Reticulocytes Express the a4b1 Integrin Complex and Bind to TNF-α Activated Endothelial Cells via a VCAM-1/$\alpha 4\beta 1$ Dependent Mechanism," 18th Annual Meeting of the National Sickle Cell Disease Program, Philadelphia, PA (May 1993).

7. Inventions and Patents

None.

PROGRESS REPORT *(Personnel and Study Subjects)*

GRANT NUMBER
HL-44960-03

All Personnel for the Current Budget Period
and Any Planned Changes in Personnel for the Next Budget Period
Use two sections. In the first section list *All Current Personnel*. In the second section list *Planned Personnel Changes*.

Name	Degree(s)	SSN	Role on Project *(e.g., PI, Res. Assoc.)*	Date of Birth *(MM/DD/YY)*	Annual % Effort
Current Personnel					
Timothy M. Wick	B.S.,Ph.D.	505-94-2891	PI	07/09/61	20%
James R. Eckman	B.A.,M.D.	471-48-8946	Co-Investigator	08/25/43	5%
Henri A. Brittain*	B.S.,M.S., Ph.D.	264-53-1153	Graduate Student	01/18/59	100%
Anjali Kumar	B.S.	253-81-0772	Graduate Student	09/15/68	100%

Planned Changes

None.

Mr. Brittain graduated in August 1992 and is no longer working on the project. Ms. Kumar is his replacement and has been working on the project since January 1993.

Provide the number of subjects enrolled in the study *to date* according to the following categories. (See Page 8 for definitions.)

	American Indian or Alaskan Native	Asian or Pacific Islander	Black, not of Hispanic Origin	Hispanic	White, not of Hispanic Origin	Other or Unknown	TOTAL
Female			56		12		68
Male			82		9		91
Unknown							
TOTAL			138		21		159

CHECKLIST

GRANT NUMBER
HL44960-03

Check the appropriate boxes and provide the information requested. Make this page the last page of the signed original of the application.

1. ASSURANCES/CERTIFICATIONS

The following assurances/certifications are made by checking the appropriate boxes and verified by the signature of the OFFICIAL SIGNING FOR APPLICANT ORGANIZATION on the FACE PAGE of the application. Descriptions of individual assurances/certifications begin on page 9 of Specific Instructions.

a. Human Subjects *(Complete Item 7 on the Face Page)*
[X] Full IRB Review [ ] Expedited Review
Use of Human Subjects [ ] Change [X] No Change Since Previous Submission

b. Vertebrate Animals *(Complete Item 8 on the Face Page)*
Use of Vertebrate Animals [ ] Change [ ] No Change Since Previous Submission

c. Inventions and Patents *(Complete Item 11 on the Face Page)* None

d. Debarment and Suspension [X] No [ ] Yes *(Attach explanation)*

e. Lobbying
With Federal appropriated funds [XXX] No
With other than Federal appropriated funds [XXX] No [ ] Yes
(If "yes", see page 13, and attach Standard Form LLL, "Disclosure of Lobbying Activities," to the application behind the Checklist.)

f. Delinquent Federal Debt [X] No [ ] Yes *(Attach explanation)*

g. Misconduct in Science (Form PHS 6315) [X] Filed [ ] Not Filed
If filed, date of Initial Assurance or latest Annual Report 1/14/92

h. Civil Rights	i. Handicapped Individuals	j. Sex Discrimination	k. Age Discrimination
Form HHS 441	Form HHS 641	Form HHS 639-A	Form HHS 680
[X] Filed	[X] Filed	[X] Filed	[X] Filed
[ ] Not Filed	[ ] Not Filed	[ ] Not Filed	[ ] Not Filed

2. PROGRAM INCOME *(See Instructions. Page 16)*

All applications must indicate (Yes or No) whether program income is anticipated during the period(s) for which grant support is requested. [X] No [ ] Yes. If "Yes" use the format below to reflect the amount and source(s) of anticipated program income.

Budget Period	Anticipated Amount	Source(s)

3. INDIRECT COST

Indicate the applicant organization's most recent indirect cost rate established with the appropriate DHHS Regional Office, or, in the case of forprofit organizations, the rate established with the appropriate PHS Agency Cost Advisory Office. Indirect costs will not be paid on foreign grants, construction grants, grants to Federal organizations and grants to individuals, and usually not on conference grants. Follow any additional instructions provided for Research Career Development Awards, Institutional National Research Service Awards, and specialized grant applications.

[ ] DHHS Agreement Dated: _____ [ ] No Indirect Costs Requested
[X] No DHHS Agreement, but rates established with Office of Naval Research Date 8/26/92

CALCULATION*

Enter proposed budget period:
Amount of base $44,470 × Rate applied 0.449 % = Indirect costs $19,967
Add to total direct costs from form page 2 and enter new total on Face Page, item 10b

*Check appropriate box(es):
[ ] Salary and wage base [X] Modified total direct costs base [ ] Other base (Attach explanation)
[ ] Off-site, other special rate, or more than one rate involved *(Attach explanation)*